

THE PATENTS ACT, 1970.



IT IS HEREBY CERTIFIED that the annexed hereto is a true copy of the Complete Specification in respect of Patent Application No. 827/Cal/1999 filed on the 6th day of October, 1999 in the name of TORRENT PHARMACEUTICALS LTD., of Central Plaza, 1st floor, Room - 106, 2/6, Sarat Bose Road, Calcutta 700 020, West Bengal, India and also at "TORRENT HOUSE" Near Dinesh Hall, Off. Ashram Road, Ahmedabad - 380009 Gujarat, India, an Indian Company.

Witness my hand: This the 27th day of December, 1999.
This the 6th day of Paus 1921 (Saka)

K. K. Modak

(K. K. MODAK)

Asstt Controller of Patents & Designs

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827/C/99

FORM 2

THE PATENTS ACT, 1970
(39 of 1970)

COMPLETE SPECIFICATION
(See Section 10)

*Drug (Product)
WTO*

TITLE

COMPOUNDS FOR THE TREATMENT OF DIABETIC AND
AGING-RELATED VASCULAR COMPLICATIONS

APPLICANT

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AN INDIAN COMPANY.

The following specification particularly describes the
nature of the invention and the manner in which it is to be
performed.

COMPOUNDS FOR THE TREATMENT OF DIABETIC AND AGING RELATED VASCULAR COMPLICATIONS

FIELD OF THE INVENTION

The present invention relates to a class of compounds of pyridinium series and to their use in treatment of diabetes and related illnesses. More particularly the invention relates to compounds of this series, methods for their preparation, pharmaceutical composition containing these compounds and their use in the treatment of complications of diabetes mellitus. The compounds of this series exhibit AGE breaking activity, which is essential for the treatment of diabetic and aging-related complications including kidney disease, nerve damage, atherosclerosis, retinopathy and dermatological conditions. The invention also extends to the method of reversing the discoloration of teeth resulting from nonenzymatic browning in the oral cavity which comprises administration of an amount effective to reverse pre-formed advanced glycosylation crosslinks.

BACKGROUND OF THE INVENTION

Maillard in 1912 found that reducing sugars, such as glucose and ribose react with proteins to form brown pigments. Further studies have shown that this is an irreversible non-enzymatic reaction, which occurs in several natural systems including stored foodstuff. Maillard reaction occurs in two stages, early and

5 advanced. Initially, proteins react with glucose to form stable Amadori products, which subsequently cross-links to form advanced glycation end products (AGE). In most cases, the formation of AGE also accompanies browning of the proteins and increase in the fluorescence.

10 In diabetes, where blood glucose level is significantly higher than normal, the reaction of glucose with several proteins such as haemoglobin, lens crystallin and collagen, gives rise to the formation of AGE, which in turn, is responsible for the complications associated with diabetes, such as nephropathy, microangiopathy, endothelial dysfunction and other organ dysfunctions. In addition, the activity of several growth factors, such as basic fibroblast growth factor, is also impaired. AGE products, unlike normal proteins in tissue, have a
15 slower rate of turnover and replenishment. It has been reported that AGE products may in fact elicit a complex immunological reaction involving RAGE (Receptor for Advanced Glycation End Products) receptors and activation of several incompletely defined immunological processes. It has been documented
20 that diabetes with evidence of microangiopathy and macroangiopathy also show evidence of oxidative stress, the mechanism of which has not been elucidated.

In vitro AGE formation can be studied in the laboratory by incubating reducing sugars, such as ribose or glucose with bovine serum albumin. AGE formation can be detected by increase in the fluorescence or increased cross

5 reactivity with anti-AGE antibodies. The increase in fluorescence seems to precede formation of AGE specific antigenic epitopes. This increase in fluorescence is used to monitor the increased AGE formation in vitro (Brownlee M et al, Science 1986; 232:1629-1632). In addition to the increase in the fluorescence, one of the most important features of in vitro AGE formation is the formation of antigenic epitopes that are specific to AGE and not to the native proteins. Therefore, it is possible to raise antibodies against advanced glycation end products of one protein and use them to detect AGE formation in other proteins. This has served as an important analytical tool in AGE research.

15 Due to the clinical significance of AGE formation, many approaches are being used to diagnose, prevent, or revert AGE formation in the body. The formation of AGE could be inhibited by reacting with an early glycosylation product that results from the original reaction between the target protein and glucose. The inhibition was believed to take place as the reaction between the inhibitor and the early glycosylation product appeared to interrupt the subsequent reaction of the glycosylated protein with additional protein material to form the cross linked late stage product. Compounds like aminoguanidine act to inhibit AGE formation by such mechanism.

20 The formation of AGE on long-lived proteins is also associated with cross-linking of these proteins. The AGE derived protein cross-links have been shown

5 to be cleaved by compounds like N- phenacyl thiazolium bromide (PTB), which
reacts with and cleaves covalent, AGE derived protein cross links (Vasan et al.
Nature 1996; 382: 275-278; US 5,853,703, Date of Patent : Dec. 29, 1998). The
mechanism of reducing the AGE content in tissues is expected to take place
relatively rapidly, in contrast to aminoguanidine, which acts slowly by its very
10 nature of mechanism of action. This current specification is related to compounds
of pyridinium class, which break pre-formed AGE, like PTB, and in some cases
even more effectively than PTB.

SUMMARY OF THE INVENTION

15 The main objective of the present invention is to provide a class of
compounds of the pyridinium series which are useful for the management of
diabetes and aging-related vascular complications, and particularly in the
treatment of complications of diabetes mellitus and other aging-related conditions
including kidney disease, nerve damage, atherosclerosis, retinopathy and
20 dermatological conditions. The invention also extends the method to reverse the
discoloration of teeth resulting from nonenzymatic browning in the oral cavity
which comprises administration of an amount effective to reverse the pre-formed
advanced glycosylation crosslinks etc.

25 Another object of the present invention is to provide compounds of the
pyridinium series, which exhibit AGE breaking activities.

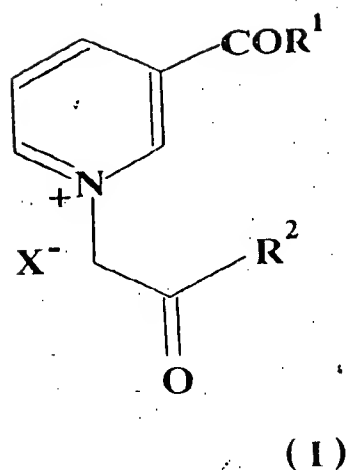
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Yet another object of the present invention is to provide a method of preparation of compounds of the pyridinium series which exhibit AGE breaking activities.

10 Still another object of the invention is to provide pharmaceutical compositions with a new class of compounds of the pyridinium series according to the invention and their pharmaceutically acceptable salts in combination with suitable carriers, solvents, excepients, diluents and other media normally employed in preparing such compositions.

15 Still another object of the invention is to provide a method of treatment of a diabetic patient by administration of the compounds of the invention, either singly or in combination with drugs for anti-diabetic therapy, or pharmaceutically acceptable salts thereof in required dosage in admixture with pharmaceutically acceptable diluent, solvent, excepients, carriers or other media as may be appropriate for the purpose.

20
DETAILED DESCRIPTION OF THE INVENTION

25 The present invention provides for a new class of AGE breakers, of general formula I,



wherein

R^1 is $-Y-R^3$

Y is selected from oxygen or NH.

R^3 is selected from hydrogen, alkyl, aryl

R^2 is selected from group consisting of alkyl, -Oalkyl, aryl, -Oaryl, -NHalkyl and -NHaryl

X is selected from group consisting of a halide ion, acetate ion, perchlorate ion, sulfonate ion, oxalate ion, citrate ion, tosylate ion, maleate ion, mesylate ion, carbonate ion, sulfite ion, phosphoric hydrogen ion, phosphonate ion, phosphate ion, BF_4^- , PF_6^- , etc.

with proviso that,

(a) when R^1 is phenyl, then R^2 is also phenyl, and

(b) when R^2 is phenyl and X is halide ion, then R^1 is other than $-OCH_3$.

As used herein "alkyl" refers to an optionally substituted, saturated hydrocarbon group joined by single carbon-carbon bonds and having 1-4 carbon

atoms joined together. The saturated alkyl hydrocarbon group may be linear or branched. The substituent is -OH.

As used herein "aryl" refers to an optionally substituted aromatic group with atleast one ring having a conjugated pi- electron system, containing upto two conjugated or fused ring systems. Aryl includes carbocyclic aryl and heterocyclic aryl all of which may be optionally substituted with halogen, sulfonamido, thio, amino, cyano, -Oalkyl, -NHalkyl, hydroxy, formyl, -Oaryl and -NHaryl.

The representative compounds of general formula I which are novel are listed in Table IA. These compounds with the following chemical names are suggested by way of example alone and in no way restrict the invention.

3-Carbonylamino-1-(2-(2,4-dichlorophenyl)-2-oxoethyl)) pyridinium bromide (compound 1).

3-(Tetrahydrobenzothiazol-2-yl)aminocarbonyl-1-(2-(2,4-dichlorophenyl)-2-oxoethyl)pyridinium bromide (compound 2).

1-(2-Phenyl-2-oxoethyl)-3-((2-hydroxyethyl)aminocarbonyl) pyridinium bromide (compound 3).

3-Carbonylamino-1-(2-thien-2'-yl-2-oxoethyl)pyridinium bromide (compound 4).

1-(2-Phenyl-2-oxoethyl)-3-((p-sulfonamidophenylene)aminocarbonyl) pyridinium bromide (compound 5).

5 1-(2-Ethoxy-2-oxoethyl)-3-((2-hydroxyethyl)aminocarbonyl)pyridinium bromide.
(compound 6).

1-(2-Phenyl - 2- oxoethyl) -3-(isopropylloxycarbonyl) pyridinium bromide
(compound 7).

10 1-(2-Methyl-2-oxoethyl)-3-((2-hydroxyethyl)aminocarbonyl) pyridinium chloride
(compound 8).

1-(2-Thien-2'-yl-2-oxoethyl)-3-((2-hydroxyethyl)aminocarbonyl)pyridinium
bromide (compound 9).

1-(2-(2,4 - Dichlorophenyl-2-oxoethyl) -3- (isopropylloxycarbonyl) pyridinium
bromide (compound 10).

15 1- (2-Phenyl - 2- oxoethyl) - 3- ((4-methylthiazol - 2 -yl) aminocarbonyl)
pyridinium bromide (compound 11).

1-(2-Phenylamino-2-oxoethyl)-3-(n-butyloxycarbonyl)pyridinium chloride
(compound 12).

20 1-(2- Phenylamino - 2- oxoethyl) - 3 - (n-butylaminocarbonyl) pyridinium
chloride (compound 13).

1- (2- Phenylamino - 2- oxoethyl)- 3- ((2-hydroxyethyl)aminocarbonyl)
pyridinium chloride (compound 14).

1- (2- (2,4 - Dichlorophenyl) - 2-oxoethyl) - 3- (n - butoxycarbonyl) pyridinium bromide (compound 15).

1-(2 - (2, 4 - Dichlorophenyl) - 2-oxoethyl) - 3- (n-butylamino-carbonyl) pyridinium bromide (compound 16).

Further, some known pyridine derivatives have also been studied for their AGE breaking activity, which was not known earlier for these molecules, and are as listed in Table 1B, with their chemical names as given below:

1- (2-Phenyl-2-oxoethyl) -3- (1-phenyl-1-oxomethyl) pyridinium bromide (compound 17 : (Ref: Chemical Abstracts: 91, P47378Y, (1979))

1- (2 Phenyl - 2-oxoethyl) - 3- (methoxycarbonyl) pyridinium bromide (compound 18 : (Ref: Chemical Abstracts: 91, P47378Y, (1979))

Table 1A – Pyridinium derivatives (Novel)

Compound No.	-R ¹	-R ²	-X
1	NH ₂	2,4-dichlorophenyl	Br
2	tetrahydrobenzo-thiazol-2-yl-amino	2,4-dichlorophenyl	Br
3	NHCH ₂ CH ₂ OH	Phenyl	Br
4	NH ₂	2-thienyl	Br
5	(p-sulfonamido-phenylene)amino	Phenyl	Br
6	NHCH ₂ CH ₂ OH	OEt	Br
7	OCH(CH ₃) ₂	Phenyl	Br
8	NHCH ₂ CH ₂ OH	CH ₃	Cl
9	NHCH ₂ CH ₂ OH	2-thienyl	Br
10	OCH(CH ₃) ₂	2,4-dichlorophenyl	Br
11	(4-methylthiazol-2-yl)amino	Phenyl	Br
12	OCH ₂ CH ₂ CH ₂ CH ₃	NHPh	Cl
13	NHCH ₂ CH ₂ CH ₂ CH ₃	NHPh	Cl

14	NHCH ₂ CH ₂ OH	NHPh	Cl
15	OCH ₂ CH ₂ CH ₂ CH ₃	2,4-dichlorophenyl	Br
16	NHCH ₂ CH ₂ CH ₂ CH ₃	2,4-dichlorophenyl	Br

Table IB - Pyridinium Derivatives (Known)

Compound No.	-R ¹	-R ²	-X
17	Phenyl	Phenyl	Br
18	OCH ₃	Phenyl	Br

According to one embodiment of the present invention, the present compounds are used for the treatment of diabetic complications, aging-related complications including kidney disease, nerve damage, atherosclerosis, retinopathy, dermatological conditions and colouration of teeth occurring due to the higher levels of preformed AGE. The increased levels of preformed AGE can be brought under control by breaking the AGE products using compounds mentioned in the invention.

The invention also provides a process for the preparation of compounds of the pyridinium series.

The said process for the preparation of compound 1, comprises, adding a solution of 2,4- dichlorophenacyl bromide in toluene to nicotinamide dissolved in refluxing toluene, refluxing for seven and half hours, cooling, filtering the precipitated solid, dissolving in methanol, treating with activated charcoal,

concentrating under vacuum, cooling in ice-salt mixture, filtering the precipitated solid and washing with methanol to obtain the desired compound.

Similarly, the other compounds of general formula I, are prepared from properly substituted pyridine derivatives followed by quaternization with appropriate reagent by refluxing in alcoholic solvents like methanol, ethanol, propanol, etc., and high boiling solvents like, toluene or xylene for 6 - 48 hrs. to give the desired compounds.

The in vitro AGE formation, studied in the laboratory, by incubating reducing sugar ribose, with protein bovine serum albumin, resulted in browning of solution and increase in the fluorescence. Fluorescence was used as the criteria to monitor the increased AGE formation.

Example 1

AGE breaker activity has been confirmed by the screening procedure as mentioned below:

Materials:

Bovine serum albumin (fraction V) (BSA)

Ribose, analytical grade

Phosphate buffered saline (PBS)

Equipment:

Microplate ELISA Reader - Spectramax Plus (Molecular Devices, USA)

Microplate washer, (Bio -Tec Instruments, USA)

pH meter

Methods of experiment:

160 mg/ml of protein, bovine serum albumin, BSA and 1.6M glucose sugar were dissolved in phosphate buffered saline, PBS. Sodium azide was added at 0.02% concentration as a preservative. The solution was filtered aseptically through a 0.22 μ M filter and kept for aging at 37°C for 16 weeks. After 16 weeks the solution was dialyzed against PBS, aliquoted and stored at -20°C.

To determine the AGE breaking activity, 10 μ g/ml and 100 μ g/ml of the 16 weeks AGE-BSA was incubated with different concentrations of the test compounds at 37°C for 24 hours and AGE breaking activity of the test compounds by ELISA was determined.

ELISA was performed as follows:

1. Different concentrations of 16 weeks AGE-BSA were coated on a microtitre plate as standard. Each concentration is coated in triplicates.
2. The test samples were coated on microtitre plate at a concentration of 5 ng. to 20 ng per well in triplicates.
3. The plate was incubated at 37°C for one hour.
4. After incubation the plate was washed with PBST (PBS with 0.05% Tween 20).

5. Blocking with 5% skimmed milk in PBS at 37°C for one hour was done.
6. The plate was washed with PBST.
7. Primary antibody against AGE-BSA was added and the plate is incubated at 37°C for one hour.
8. The plate was washed with PBST.
9. Secondary antibody anti rabbit HRPO (Horse-Radish Per Oxidase) conjugate was added and the plate is incubated at 37°C for one hour.
10. The plate was washed with PBST.
11. Colour development with OPD (orthophenylenediamine dihydrochloride) and hydrogen peroxide was done.
12. OD (optical density) at (450nm reading - 620nm reading) was measured after incubation at 37°C for 15 minutes with Microplate ELISA Reader.

The breaker activity of the compounds were determined by the following formula:

$$\% \text{ Breaker activity} = \frac{\text{OD}_{450-620} \text{Control} - \text{OD}_{450-620} \text{Test}}{\text{OD}_{450-620} \text{Control}} \times 100$$

$\text{OD}_{450-620} \text{Control}$ = Absorbance of 20ng AGE-BSA after incubation at 37°C for 24 hours without test compound

OD₄₅₀₋₆₂₀ Test = Absorbance of 20ng AGE-BSA after incubation at 37°C for 24 hours with required concentration of test compound

Using specific examples, the % AGE breaking activity was calculated and recorded in Table 2.

Table 2

Sample	Concentration	%Breakage
PTB	10 mM	27
	20 mM	47
Compound 3	10 mM	5
Compound 4	10 mM	3
Compound 6	10 mM	43
Compound 9	10 mM	50
Compound 12	10 mM	57
Compound 13	10 mM	27
Compound 14	20 mM	48
Compound 17	5 mM	45
Compound 18	20 mM	36

Hence, for example, compound 12 has significant AGE - breaking activity i.e. a comparatively much superior potency vis - a - vis PTB.

The following examples give method for preparation of the specific compounds of the invention as given in Table 1. The following compounds suggested are by way of example alone and in no way restrict the invention.

Example 2

Preparation of 3- carbonylamino -1- (2- (2,4-dichlorophenyl) -2- oxoethyl) pyridinium bromide (compound 1)

Nicotinamide (1.22g, 0.01 mol) was dissolved in refluxing toluene (40 ml) and a solution of 2,4-dichlorophenacyl bromide (3.0g, 0.012mol) in 10ml of toluene was added. The reaction mixture was refluxed for 7.5 hours and cooled. The precipitated solid was filtered and dissolved in methanol, decolourized with activated charcoal and concentrated under vacuum to one-fourth volume. It was cooled in ice - salt mixture and the precipitated solid was filtered and washed with methanol (3x10ml) to afford a pure solid.

Yield : 39%

m.p. : 237-239°C

IR(KBr,cm⁻¹) : 3331, 3133, 1706, 1678

¹H NMR (DMSOd₆, 400 MHz) δ : 9.54(1H,s), 9.18-9.11(2H,m), 8.67(1H,s), 8.40(1H,t), 8.42-8.38(2H,m), 7.88(1H,s), 7.75 -7.72(1H,m), 6.49(2H,s)

Mass (m/z): 309,310,311,312,187,159

According to the above mentioned procedure the following compounds are synthesized by reacting the corresponding pyridine derivatives with appropriate reagents by refluxing in alcoholic solvents like methanol, ethanol, propanol, etc. and high boiling solvents like toluene or xylene for 6-48 hours to give the desired compounds:

Example 3

3-(Tetrahydrobenzothiazol-2-yl)aminocarbonyl)-1-(2-(2,4-dichlorophenyl)-2-oxoethyl) pyridinium bromide (compound 2):

Yield : 48%

m.p. : 165 - 167 °C(decomp.)

IR(KBr,cm⁻¹) : 3333, 1714, 1684, 1635

¹H NMR(CD₃OD, 400MHz) δ: 9.45(1H,s), 9.27-9.24(1H,m), 8.92-8.91(1H, m), 8.24 - 8.21(1H, m), 8.01 - 7.99(1H, m), 7.72 - 7.71(1H, m) 7.57-7.54(1H,m), 2.59-2.57 (4H,m), 1.85(4H,m)

Mass (m/z) : 446, 447, 448, 449, 416, 307 and 266

Example 4

1-(2- Phenyl -2- oxoethyl) -3- ((2- hydroxyethyl) aminocarbonyl) pyridinium bromide (compound 3):

Yield : 98%

m.p. : 182 - 184°C(decomp.)

IR(KBr,cm⁻¹) : 3289, 3241, 1690 and 1660

¹H NMR(DMSO-d₆, 400MHz) δ: 9.47(1H,s), 9.21(1H,t), 9.09(2H,t), 8.41-8.37(1H,m), 8.08-8.04(2H,m), 7.82-7.78(1H,m), 7.69-7.65(2H,m), 6.52(2H,s), 4.86(1H,t), 3.58-3.54(2H,m), 3.42-3.38(2H,m)

Mass (m/z) : 285,242,149,119,91

Example 5

3-Carbonylamino-1- (2-thien-2'-yl-2-oxoethyl) pyridinium bromide
(compound 4).

Yield : 35%

m.p. : 212 - 215°C (decomp.)

IR(KBr,cm⁻¹) : 3295, 3126, 1680, 1671, 1640

¹H NMR (DMSO-d₆, 400 MHz) δ: 9.49(1H,s), 9.13-9.11(1H,d), 9.07-9.05(1H,d), 8.60(1H,bs), 8.40-8.38(1H,m), 8.25-8.19(3H,m), 7.43-7.40,(1H,t), 6.44(2H,s)

Mass(m/z) : 247,248,249,193

Example 6

1- (2-Phenyl -2- oxoethyl) -3- ((p-sulfonamidophenylene). aminocarbonyl)
pyridinium bromide (compound 5):

Yield : 44%

m.p. : 188-190°C

IR(KBr, cm^{-1}) : 3296, 1700, 1679.

^1H NMR (DMSO-d_6 , 400MHz) δ : 11.25 (1H,s), 9.58 (1H,s), 9.25 (1H,d), 9.16 (1H, d), 8.45 (1H,t), 8.10 (2H,d), 7.94 (2H,d), 7.86 (2H,d), 7.82(1H,t), 7.68(2H,t), 7.36(2H,s), 6.5(2H,s)

Mass (m/z) : 396, 277

Example 7

1- (2- Ethoxy -2- oxoethyl) -3- ((2- hydroxyethyl) aminocarbonyl) pyridinium bromide (compound 6):

Yield : 87%

m.p. : 138-140°C

IR(KBr, cm^{-1}) : 1748, 1669

^1H NMR (CD_3OD , 400 MHz) δ : 9.43 (1H,s), 9.09-9.02 (2H,m), 8.26 (1H,m), 5.64 (2H,s), 4.31 (2H,q), 3.73 (2H,t), 3.54 (2H,t), 1.32 (3H,t)

Mass (m/z) : 251, 252, 165, 166

Example 8

1- (2- Phenyl -2- oxoethyl) -3- (isopropyloxycarbonyl) pyridinium bromide (compound 7):

Yield : 46%

m.p. : 172-174°C

IR(KBr, cm^{-1}) : 1726, 1692

^1H NMR (DMSO-d_6 , 400MHz) δ : 9.55 (1H,s), 9.16 (1H,d), 9.08(1H,d), 8.39-8.36 (1H,m), 8.04 (2H,d), 7.77 (1H,t), 7.64 (2H,t), 6.53 (2H,s), 5.25-5.19 (1H,m), 1.34 (6H,d)

Mass (m/z) : 284, 285, 242

Example 9

1- (2- Methyl-2-oxoethyl) -3- ((2- hydroxyethyl) aminocarbonyl) pyridinium chloride (compound 8):

Yield : 47%

m.p. : 178-180°C

IR(KBr, cm^{-1}) : 1727, 1660

^1H NMR (DMSO-d_6 , 400 MHz) δ : 9.33 (1H,t), 9.30 (1H,s), 9.06(1H,d), 8.90 (1H,d), 8.25-8.21 (1H,m), 5.75 (2H,s), 4.84(1H,bs), 3.47 (2H,t), 3.30 (2H,t), 2.23 (3H,s)

Mass (m/z) : 223, 224, 225

Example 10

1- (2- Thien -2'-yl -2- oxoethyl) -3- ((2- hydroxyethyl) aminocarbonyl) pyridinium bromide (compound 9):

Yield : 60%

m.p. : 207-209°C

IR(KBr, cm^{-1}) : 1673, 1656

¹H NMR (DMSO-d₆, 400MHz) δ: 9.47 (1H,s), 9.18-9.05 (3H,m), 8.38-8.34 (1H,m), 8.23-8.19 (2H,m), 7.39 (1H,t), 6.44 (2H,s), 3.55-3.50 (2H,m), 3.40-3.37 (2H,m)

Mass (m/z) : 291, 292, 293

Example 11

1- (2- (2,4- Dichlorophenyl) -2- oxoethyl) -3- (isopropoxy carbonyl) pyridinium bromide (compound 10):

Yield : 26%

m.p. : 160-162°C

IR (KBr, cm⁻¹) : 1726, 1705

¹H NMR (DMSO-d₆, 400 MHz) δ: 9.55 (1H,s), 9.15 (1H,d), 9.08(1H,d), 8.40-8.36 (1H,m), 8.11 (1H,d), 7.89 (1H,bs), 7.75-7.72 (1H,m), 6.44 (2H,s), 5.26-5.20 (1H,m), 1.34 (6H,d).

Mass (m/z) : 352, 353, 354, 310

Example 12

1- (2- Phenyl -2- oxoethyl) -3- ((4- methylthiazol -2- yl) aminocarbonyl) pyridinium bromide (compound 11):

Yield : 30%

m.p. : 165-167°C

IR (KBr, cm^{-1}) : 3409; 3319 and 1698

^1H NMR (DMSO-d_6 , 400 MHz) δ : 9.58 (1H,s), 9.22 (1H,d), 9.11(1H,d), 8.42-8.38 (1H,m), 8.07 (2H,d), 7.81 (1H,t), 7.68(2H,t), 6.86 (1H,bs), 6.56 (2H,s), 2.30 (3H,s)

Mass (m/z) : 337, 338, 232, 105.

Example 13

1- (2- Phenylamino -2- oxoethyl) -3- (n- butoxycarbonyl) pyridinium chloride

(compound 12):

Yield : 10%

m.p. : 150-152°C

IR (KBr, cm^{-1}) : 3228, 1742, 1678 (bs)

^1H NMR (DMSO-d_6 , 400 MHz) δ : 10.96 (1H,s), 9.65 (1H,s), 9.28(1H,t), 9.09 (1H,d), 8.37 – 8.34 (1H,m), 7.62 – 7.59 (2H,m), 7.37 – 7.33 (2H,m), 7.11 (1H,t), 5.79 (2H,s), 4.41(2H,t), 1.76-1.72(2H,m), 1.48-1.43 (2H,m), 0.94 (3H,t)

Mass (m/z) : 314, 315

Example 14

1- (2- Phenylamino -2- oxoethyl) -3- (n- butylaminocarbonyl) pyridinium chloride (compound 13):

Yield : 37%

m.p.: 182-185°C

IR (KBr, cm^{-1}) : 3245, 1742, 1679

^1H NMR (DMSO-d_6 , 400MHz) δ : 10.97 (1H,s), 9.50(1H,s), 9.24(1H,t), 9.13 (1H,d), 9.02 (1H,d), 8.28-8.25 (1H,m), 7.57 (2H,d), 7.30(2H,t), 7.05 (1H,t), 5.70 (2H,s), 3.30 – 3.26 (2H,m), 1.52 – 1.48 (2H,m), 1.34 – 1.30 (2H,m), 0.86 (3H,t)

Mass (m/z) : 312, 313

Example 15

1- (2- Phenylamino -2- oxoethyl) -3- ((2- hydroxyethyl) aminocarbonyl) pyridinium chloride (compound 14):

Yield : 58%

m.p. : 225-227°C

IR (KBr, cm^{-1}) : 3448, 3271, 1702 and 1663

^1H NMR (DMSO-d_6 , 400 MHz) δ : 11.07 (1H,s), 9.58 (1H,s), 9.35(1H,t), 9.17 (1H,d), 9.11 (1H,d), 8.33-8.29 (1H,m), 7.60(2H,d), 7.32 (2H,t), 7.08 (1H,t), 5.75 (2H,s), 4.90 (1H,t), 3.57-3.53 (2H,m), 3.40-3.36 (2H,m)

Mass (m/z) : 300, 301, 302

Example 16

1- (2- (2,4- Dichlorophenyl) -2- oxoethyl) -3- (n- butoxycarbonyl) pyridinium bromide (compound 15):

Yield : 38%

m.p. : 154-156°C

IR(KBr, cm^{-1}) : 3435, 3389, 1731 and 1704

^1H NMR (DMSO-d_6 , 400 MHz) δ : 9.60 (1H,s), 9.21 (1H,d), 9.14(1H,d), 8.43 (1H,t), 8.16 (1H,d), 7.92 (1H,s), 7.78-7.76(1H,m), 6.51 (2H,s), 4.42 (2H,t), 1.76-1.72 (2H,m), 1.48-1.42 (2H,m), 0.94 (3H,t)

Mass (m/z) : 366, 367, 368, 369, 370

Example 17

1- (2- (2,4- Dichlorophenyl) -2- oxoethyl) -3- (n-butylaminocarbonyl) pyridinium bromide (compound 16):

Yield : 35%

m.p. : 142-144°C

IR(KBr, cm^{-1}) : 3382, 1698, 1672

^1H NMR (DMSO-d_6 , 400 MHz) δ : 9.37 (1H,s), 9.07 (1H,t), 8.99(2H,t), 8.31-8.28 (1H,m), 8.04 (1H,d) 7.82-7.81 (1H,d), 7.68-7.65 (1H,m), 6.34(2H,s), 3.27-3.24 (2H,m), 1.47-1.43 (2H,m), 1.29-1.24 (2H,m), 0.81 (3H,t)

Mass (m/z) : 365, 366, 367, 368, 369

Pharmaceutical Compositions

Pharmaceutical compositions may be prepared with a pharmaceutically effective quantity of compounds of general formula I, individually or in combination. The following pharmaceutical formulations suggested are by way of example alone and in no way restrict the forms in which they can be used.

Oral formulations

Oral formulations may be administered as solid dosage forms for example pellets, powders, sachets or discrete units such as tablets or capsules and like. Other orally administered pharmaceutical preparations include monophasic and biphasic liquid dosage forms either in ready to use form or forms suitable for reconstitution such as mixtures, syrups, suspensions or emulsions. The preparations in addition may contain diluents, dispersing agents, buffers, stabilizers, solubilizers, surfactants, preservatives, chelating agents and/ or other pharmaceutical additives as are used. Aqueous or non-aqueous vehicle or their combination may be used and if desired may contain suitable sweetener, flavoring agent or similar substances. In case of suspension or emulsion a suitable thickening agent or suspending agent or emulsifying agent may be present in addition. Alternatively, the compounds may be administered as such in their pure form unassociated with other additives for example as capsules or sachets. It may also be administered with a vehicle. Pharmaceutical preparations can have a slow, delayed or controlled release of active ingredients as is provided by a matrix or diffusion controlled system.

When the present invention or its salts or suitable complexes is presented as a discrete unit dosage form like a tablet, it may contain in addition medically inert excipients as are used in the art. Diluents such as starch, lactose, dicalcium

phosphate, talc, magnesium stearate, polymeric substances like methyl cellulose, fatty acids and derivatives, sodium starch glycollate, etc. may also be used.

Example 18

Preparation of oral dosage form:

A typical tablet has the following composition:

Active ingredient of formula I	as given above
Lactose	135 mg
Starch	76 mg
Polyvinyl pyrrolidone (K-30)	2 mg
Talc	1.5 mg
Magnesium Stearate	1.0 mg

Parenteral Formulations

For parenteral administration, the compounds or their salts or suitable complexes thereof may be present in a sterile vehicle which may be an aqueous or non aqueous vehicle or a combination thereof. The examples of vehicles are water, ethyl oleate, oils and derivatives of polyols, glycols and their derivatives. It may contain additives common in injectable preparations like stabilizers, solubilizers, pH modifiers, buffers, antioxidants, cosolvents, complexing agents, tonicity modifiers, etc.

Some suitable additives are for example tartrate, citrate or similar buffers, alcohol, sodium chloride, dextrose and high molecular weight polymers. Another alternative is sterile powder reconstitution. The compound may be administered in the form of injection for more than once daily administration, or intravenous infusion/ drip or suitable depot preparation.

Example 19

Preparation suitable for parenteral administration has the following composition:

Active ingredient of formula I	as given above
Polyethylene glycol (400)	0.75 ml
Sodium metabisulphite	0.01%
Isotonic saline/ WFI	q.s.

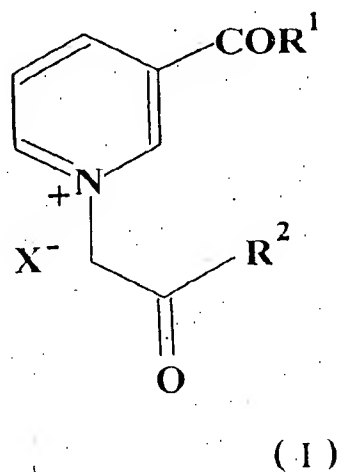
Other Formulations.

For the dermatological application and for the discoloration of teeth, the recommended formulations are lotions, oral rinse and toothpaste containing appropriate amount of the compounds of general formula I.

The above examples are presented by way of illustration alone and in no way limit the scope of the invention.

WE CLAIM:

1. A compound of pyridinium series of general formula I and its pharmaceutically acceptable salts, useful for the management of vascular complications associated with diabetes and aging-related disorders,



wherein

R^1 is $-Y-R^3$

Y is selected from oxygen or NH.

R^3 is selected from hydrogen, alkyl, aryl

R^2 is selected from group consisting of alkyl, -Oalkyl, aryl, -Oaryl, -NHalkyl and -NHaryl

X is selected from group consisting of a halide ion, acetate ion, perchlorate ion,

5 sulfonate ion, oxalate ion, citrate ion, tosylate ion, maleate ion, mesylate ion, carbonate ion, sulfite ion, phosphoric hydrogen ion, phosphonate ion, phosphate ion, BF_4^- , PF_6^- ,

with proviso that,

(a) when R^1 is phenyl then R^2 is also phenyl, and

10 (b) when R^2 is phenyl and X is a halide ion, R^1 is other than $-\text{OCH}_3$.

2. The compound as claimed in claim 1, wherein X is a halide ion.

3. The compound as claimed in claim 1, which is selected from the group consisting of the following compounds :

15 a) 1-(2-phenylamino-2-oxoethyl)-3-(n-butyloxycarbonyl) pyridinium chloride or a pharmaceutically acceptable salt thereof.

b) 1-(2-thien-2'-yl-2-oxoethyl)-3-((2-hydroxyethyl) aminocarbonyl)pyridinium bromide or a pharmaceutically acceptable salt thereof.

c) 1-(2-ethoxy-2-oxoethyl)-3-((2-hydroxyethyl) aminocarbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof.

20 d) 1-(2-phenylamino-2-oxoethyl)-3-(n-butylaminocarbonyl) pyridinium chloride or a pharmaceutically acceptable salt thereof.

e) 1-(2-phenylamino-2-oxoethyl)-3-((2-hydroxyethyl) aminocarbonyl)pyridinium chloride or a pharmaceutically acceptable salt thereof.

f) 1-(2-phenyl-2-oxoethyl)-3-((2-hydroxyethyl) aminocarbonyl)pyridinium

5 bromide or a pharmaceutically acceptable salt thereof.

g) 1-(2-methyl-2-oxoethyl)-3-((2-hydroxyethyl) aminocarbonyl)pyridinium
bromide or a pharmaceutically acceptable salt thereof.

h) 1-(2-phenyl-2-oxoethyl)-3-((4-methylthiazol-2-yl) aminocarbonyl)pyridinium
bromide or a pharmaceutically acceptable salt thereof.

10 i) 1-(2-(2,4-dichlorophenyl)-2-oxoethyl)-3-(isopropoxy carbonyl)pyridinium
bromide or a pharmaceutically acceptable salt thereof.

j) 3-(tetrahydrobenzothiazol-2-yl)aminocarbonyl)-1-(2-(2,4-dichlorophenyl)-2-
oxoethyl)pyridinium bromide or a pharmaceutically acceptable salt thereof.

15 k) 1-(2-phenyl-2-oxoethyl)-3-((p-sulfonamidophenylene)aminocarbonyl))
pyridinium bromide or a pharmaceutically acceptable salt thereof.

l) 3-carbonylamino-1-(2-thien-2'-yl-2-oxoethyl)pyridinium bromide or a
pharmaceutically acceptable salt thereof.

m) 1-(2-(2,4-dichlorophenyl)-2-oxoethyl)-3-(n-butylaminocarbonyl)pyridinium
bromide or a pharmaceutically acceptable salt thereof.

20 n) 1-(2-(2,4-dichlorophenyl)-2-oxoethyl)-3-(n-butoxycarbonyl)pyridinium
bromide or a pharmaceutically acceptable salt thereof.

4. A process for the preparation of compounds of the pyridinium series as
claimed in claim 1, wherein said process comprises preparation of the properly
substituted pyridine derivatives according to the desired end products followed

5 • by quaternization of the substituted pyridine derivatives with appropriate reagent
by refluxing in alcoholic solvents and/or high boiling solvents for 6 to 48 hrs. to
give the desired compounds.

5. The use of a compound of general formula I as defined in claim 1, in the
manufacture of a medicament for diabetic complications and aging-related
10 diseases, including kidney disease, nerve damage, retinopathy, atherosclerosis,
microangiopathy, endothelial dysfunctions, dermatological conditions,
discoloration of teeth and other organ dysfunctions.

6. The use as claimed in claim 5, wherein said compound is selected from the
group consisting of :

- 15 a) 1-(2-phenylamino-2-oxoethyl)-3-(n-butyloxycarbonyl) pyridinium chloride or
a pharmaceutically acceptable salt thereof.
- b) 1-(2-thien-2'-yl-2-oxoethyl)-3-((2-hydroxyethyl) aminocarbonyl)pyridinium
bromide or a pharmaceutically acceptable salt thereof.
- c) 1-(2-ethoxy-2-oxoethyl)-3-((2-hydroxyethyl) aminocarbonyl)pyridinium
20 bromide or a pharmaceutically acceptable salt thereof.
- d) 1-(2-phenylamino-2-oxoethyl)-3-(n-butylaminocarbonyl) pyridinium chloride
or a pharmaceutically acceptable salt thereof.
- e) 1-(2-phenylamino-2-oxoethyl)-3-((2-hydroxyethyl)aminocarbonyl) pyridinium
chloride or a pharmaceutically acceptable salt thereof.

f) 1-(2-phenyl-2-oxoethyl)-3-((2-hydroxyethyl) aminocarbonyl)pyridinium bromide or a pharmaceutically acceptable salt thereof.

g) 1-(2-methyl-2-oxoethyl)-3-((2-hydroxyethyl) aminocarbonyl)pyridinium bromide or a pharmaceutically acceptable salt thereof.

h) 1-(2-phenyl-2-oxoethyl)-3-((4-methylthiazol-2-yl) aminocarbonyl)pyridinium bromide or a pharmaceutically acceptable salt thereof.

i) 1-(2-(2,4-dichlorophenyl)-2-oxoethyl)-3-(isopropylloxycarbonyl)pyridinium bromide or a pharmaceutically acceptable salt thereof.

j) 3-(tetrahydrobenzothiazol-2-yl)aminocarbonyl-1-(2-(2,4-dichlorophenyl)-2-oxoethyl)pyridinium bromide or a pharmaceutically acceptable salt thereof.

k) 1-(2-phenyl-2-oxoethyl)-3-((p-sulfonamidophenylene)aminocarbonyl)pyridinium bromide or a pharmaceutically acceptable salt thereof.

l) 3-carbonylamino-1-(2-thien-2'-yl-2-oxoethyl)pyridinium bromide or a pharmaceutically acceptable salt thereof.

m) 1-(2-(2,4-dichlorophenyl)-2-oxoethyl)-3-(n-butylaminocarbonyl)pyridinium bromide or a pharmaceutically acceptable salt thereof.

n) 1-(2-(2,4-dichlorophenyl)-2-oxoethyl)-3-(n-butoxy carbonyl)pyridinium bromide or a pharmaceutically acceptable salt thereof.

7. The use of 1-(2-phenyl-2-oxoethyl)-3-(1-phenyl-1-oxomethyl)pyridinium bromide or a pharmaceutically acceptable salt thereof in the manufacture of a

medicament for diabetic complications and ageing related diseases including kidney disease, nerve damage, retinopathy, atherosclerosis, microangiopathy, endothelial dysfunctions, dermatological conditions, discoloration of teeth and other organ dysfunctions.

8. The use of 1-(2-phenyl-2-oxoethyl)-3-(methoxycarbonyl) pyridinium bromide or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for diabetic complications and ageing related diseases including kidney disease, nerve damage, retinopathy, atherosclerosis, microangiopathy, endothelial dysfunctions, dermatological conditions, discoloration of teeth and other organ dysfunctions.

9. A pharmaceutical composition for treatment of diabetic complications and aging related diseases which comprises a pharmaceutically effective amount of one or more compounds of general formula I, as defined in claim 1, or pharmaceutically acceptable salt(s) thereof, in admixture with a pharmaceutically acceptable carrier, diluent, solvent or excipient.

10. The pharmaceutical composition as claimed in claim 9, in the form of an oral formulation.

11. The pharmaceutical composition as claimed in claim 10, wherein the said

pharmaceutically acceptable carrier is selected from one or more of the compounds starch, lactose, polyvinylpyrrolidone (K-30), talc and magnesium stearate.

12. The pharmaceutical composition as claimed in claim 9, in the form of parenteral formulation.

13. A method for the preparation of a parenteral formulation as claimed in claim 12, which comprises dissolving the active ingredient of general formula 1, as defined in claim 1, in polyethylene glycol 400 and diluting the solution so obtained, with an isotonic solution or water to the desired concentration.

14. The pharmaceutical composition as claimed in claim 9, in the form of lotions, oral rinse and toothpaste.

15. A method for treating a diabetic patient by breaking the preformed AGE within said patient, which comprises administering an effective amount of a compound as claimed in claim 1, either singly or in combination with other drugs for antidiabetic therapy.

16. A method for treating a diabetic patient by breaking the preformed AGE within said patient which comprises, administering an effective amount of 1-(2-phenyl-2-oxoethyl)-3-(1-phenyl-1-oxomethyl)pyridinium bromide or a pharmaceutically acceptable salt thereof, either singly or in combination with other drugs for antidiabetic therapy.

¶ 17. A method for treating a diabetic patient by breaking the preformed AGE, within said patient, which comprises, administering an effective amount of 1-(2-phenyl-2-oxoethyl)-3-(methoxycarbonyl)pyridinium bromide or a pharmaceutically acceptable salt thereof, either singly or in combination with other drugs for antidiabetic therapy.

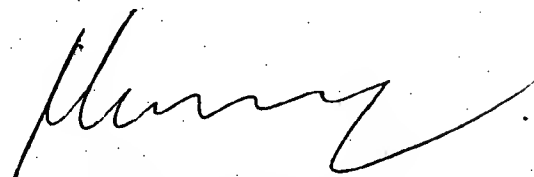
10 18. A method of preventing or treating diseases caused by diabetes and aging-related complications, which comprises, administering to a patient in need thereof, an effective amount of a compound of formula I, as claimed in claim 1, either singly or in combination with a pharmaceutically acceptable carrier, diluent or excepiant.

15 19. The method as claimed in claim 18, wherein the disease caused to be prevented or treated is a nephrological disorder, neurological disorder, atherosclerosis, retinal disorder, dermatological disorder, non-enzymatic browning of oral cavity, endothelial or other organ dysfunction and growth impairment.

20. A process for the preparation of compounds of the pyridinium series, substantially as herein described, particularly with reference to the examples.

21. A method of making a pharmaceutical composition, substantially as herein described, particularly with reference to the examples.

Dated this 6th day of October, 1999.

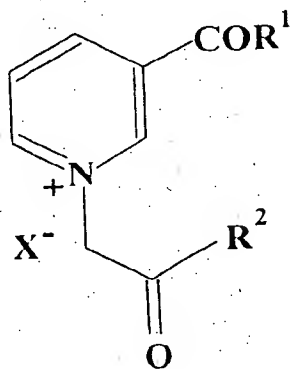


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APPLICANTS' AGENT

**"COMPOUNDS FOR THE TREATMENT OF DIABETIC AND
AGING-RELATED VASCULAR COMPLICATIONS"**

ABSTRACT

The invention discloses compounds of the pyridinium series useful for the management of diabetes and aging-related vascular complications, and particularly in the treatment of complications of diabetes mellitus and other aging-related conditions including kidney disease, nerve damage, atherosclerosis, retinopathy, dermatological conditions and discoloration of teeth by breaking pre-formed AGE, of the general formula I, or pharmaceutically acceptable salts thereof,



(I)

wherein, R^1 , R^2 and X are as defined in the specification.

The invention also discloses method of preparation of the compounds of the series and pharmaceutical composition having one or more compounds as defined above as active ingredients.

The invention further discloses a method of treatment of a diabetic patient by administering the compounds as defined above, either singly or in combination with other drugs for antidiabetic therapy.